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Genotypic Diversity of Beet Curly Top Virus

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by

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ABSTRACT:

The genotypic diversity of beet curly top virus (BCTV) present in the western United States has been examined by the analysis if 58 field isolates and eight laboratory or nursery isolates of the virus. Full-length clones for each isolate have been characterized for genotype by restriction endonuclease mapping. Only variants of the CFH and Worland strains were recovered from field isolates. Variants of the Cal/Logan strain were found only in laboratory or nursery isolates.

Introduction

Geminiviruses are plant pathogens characterized by their unusual paired-icosahedral capsids. Although they were discovered as a distinct family in 1978, the first geminivirus was not cloned and sequenced until 1983 (1). Much knowledge has been gained on them since with the advancements in recombinant DNA technology. Today, there are three subgroups of geminiviruses (1) that are differentiated on the basis of insect vector specificity, host range, and genome organization. Members of subgroup I infect monocots, while those in subgroups II and III infect dicots. Viruses in subgroups I and II are transmitted by leafhoppers (*Cicadulina*), whereas those of subgroup III are transmitted by whiteflies (*Bemisia tabaci*).

Beet curly top virus (BCTV) is the lone member of subgroup II. BCTV is leafhopper-transmitted and infects an extremely wide range of dicotyledonous hosts. Characterization of three distinct strains of BCTV has been accomplished over the past 10 years by assessing clones derived from laboratory-maintained isolates (7). CFH, Worland, and Calif/Logan strains have been distinguished based on phenotypic characteristics, genomic sequences, and virulence (7). California and Logan, originally classified as two separate strains, have been determined to be minor variants of the same strain.

The primary host for which BCTV was named is the sugar beet, which is cultivated throughout the western United States. The sugar beet industry suffers losses each year due to the affects of BCTV. The sugar beet industry is attempting to develop plants resistant or tolerant to the virus using laboratory-maintained isolates of the virus.

However, the distribution of BCTV strains in commercial sugar beet plantings is unknown. In this experiment, field isolates of BCTV collected from commercial sugar beet fields throughout the western U.S. were characterized for genotypic properties and compared to previously examined laboratory isolates.

Materials and Methods

Sample collection and extraction. Sugar beet plant samples displaying symptoms typical of BCTV (vein swelling and leaf curling) were collected from locations throughout the western United States (Fig. 1). Industrial collaborators in Washington, Wyoming, Idaho, Colorado, California, New Mexico, Texas, and Oregon provided the forty-three samples (Table 1). Total DNA (plant and viral) had been previously extracted from 5g leaf samples of each plant by Stenger using methods previously described (5). Each total DNA extraction had been adjusted to the appropriate concentration (1mg/mL) and stored at -20 C.

Identification and characterization of BCTV by Southern blot. A 5μg sample of total DNA extraction from each isolate was incubated with the restriction endonuclease *Eco*RI. The digested samples, along with native DNA, were resolved on an agarose gel. The DNA in the gel was then eluted in the same spatial configuration by capillary transfer to a nitrocellulose membrane. UV crosslinking bound the DNA tightly to the membrane. A radiolabeled RNA probe of virion-sense was transcribed with T7 RNA polymerase using pCT9 as a template and was hybridized to the DNA. pCT9 contains a genome-length

*Sal*I insert derived from pLOGAN (8). This particular probe can detect double-stranded viral DNA of all three BCTV strains (4).

Purification, cloning, and analysis of viral DNA. BCTV DNA from all samples tested was linearized by *Eco*RI digestion. A 50μg aliquot of each total DNA extraction was incubated with *Eco*RI to linearize the DNA. The digested samples were size-fractioned on agarose gels by electrophoresis, and the genome-length viral DNA (2.9 kb) was recovered from the gels using the Gene Clean II system (BIO 101, La Jolla, CA). The recovered DNA was ligated to *Eco*RI-digested pUC8 and transformed into *Escherichia coli* strain DH5α. Cells were plated on media containing ampicillin and X-gal to select for positive transformants, which were then screened for BCTV inserts by colony hybridization (2). Plasmid DNA was purified from clones identified as positive for BCTV and was characterized by restriction endonuclease mapping.

Results

Full-length clones derived from laboratory-maintained isolates of BCTV (California, Logan, CFH, and Worland) were characterized previously for their genotypic properties (6). Table 2 lists these clones along with clones from four previously described nursery isolates (7). Characterization of clones from the nursery isolates suggested that all three strains were represented among the isolates, displaying minor genotypic variability among each strain.

A total of 106 cloned genomes from the forty-three sugar beet field isolates (Table1) were restriction mapped using fifteen restriction endonucleases. Genotypic variants of

the CFH and Worland strains were found to be represented among the isolates. Clones from four pepper field isolates were also characterized and were found to contain variants of the Worland strain (Table 1). Eleven additional sugar beet field isolates previously cloned and characterized by Stenger (5) are also included in the population analyses (Table 1). Similarly, these samples were shown to have contained variants of the CFH strain.

Restriction endonuclease maps of the full-length BCTV genome of each of the aforementioned clones were constructed. Individual, distinct genotypes represented in the clones were determined to be variants of CFH (Fig. 2), Worland (Fig. 3), or Calif/Logan (Fig. 4). The genotypes were assigned numbers for the purpose of identity. The restriction maps initiate at the *Eco*R1 insertion site and are depicted linearly, displaying relative position of each restriction site in the genome. Relative positions and polarity of the seven open reading frames and the origin of replication are illustrated above each set of maps for each strain (Fig. 2, 3, 4).

Each strain showed some degree of polymorphism among the restriction sites (Table 3, Fig. 5). The CFH and Calif/Logan strains have a number of sites that are conserved among all variants found. Worland displayed relatively less site conservation. However, all three strains showed only minor variability among the individual genotypes within each strain.

Each distinct restriction site is assigned a number in Figure 5, and the frequency of the occurrence of sites among the genotypes is summarized in Table 3. Sites that were conserved within a strain show a frequency of 100%.

Although the results suggest the occurrence of only the CFH and Worland strains in the field (Table 1), there is evidence of genotypic complexity within a number of the isolates (Table 4). Of the 58 isolates analyzed, 21 of them were represented by only one clone and, consequently, only one genotype. In addition, 17 isolates produced multiple clones containing genomes of only one genotype, suggesting infection by one homogenous population of virus. However, 16 isolates produced cloned genomes of different genotypes of the same strain, and 4 isolates produced cloned genomes of different genotypes and different strains, thus demonstrating infection of the same plant by more than one virus strain.

Discussion

All CFH and Worland genotypes, other than the pWORLAND clone representing the Worland strain derived from a laboratory-maintained isolate (Table 2), were found in field isolates (Table 5). The Calif/Logan strain was not found in any of the field isolates examined in this experiment, nor has it been documented to be found in any experiments prior. This suggests the possible extinction of the Calif/Logan strain in the field. It seems to prevail only in laboratory and nursery settings where it is maintained by humans. Attempts made by the sugar beet industry to engineer resistant plants are being structured around the assumption that Calif/Logan is the predominant isolate in the field. This is clearly a false assumption. Also apparent from the results of this experiment is the genetic diversity of the CFH and Worland strains in the field. Evidently, the strategies being employed by the industry need to be reevaluated.

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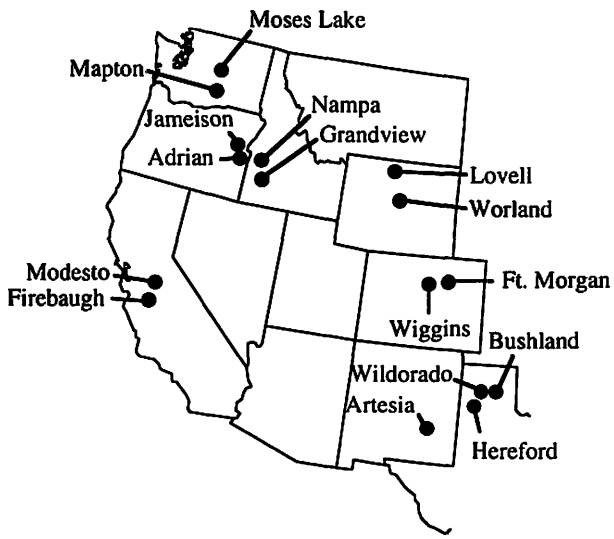

Fig. 1. Localities from which field isolates of BCTV were collected in 1994 and 1995.

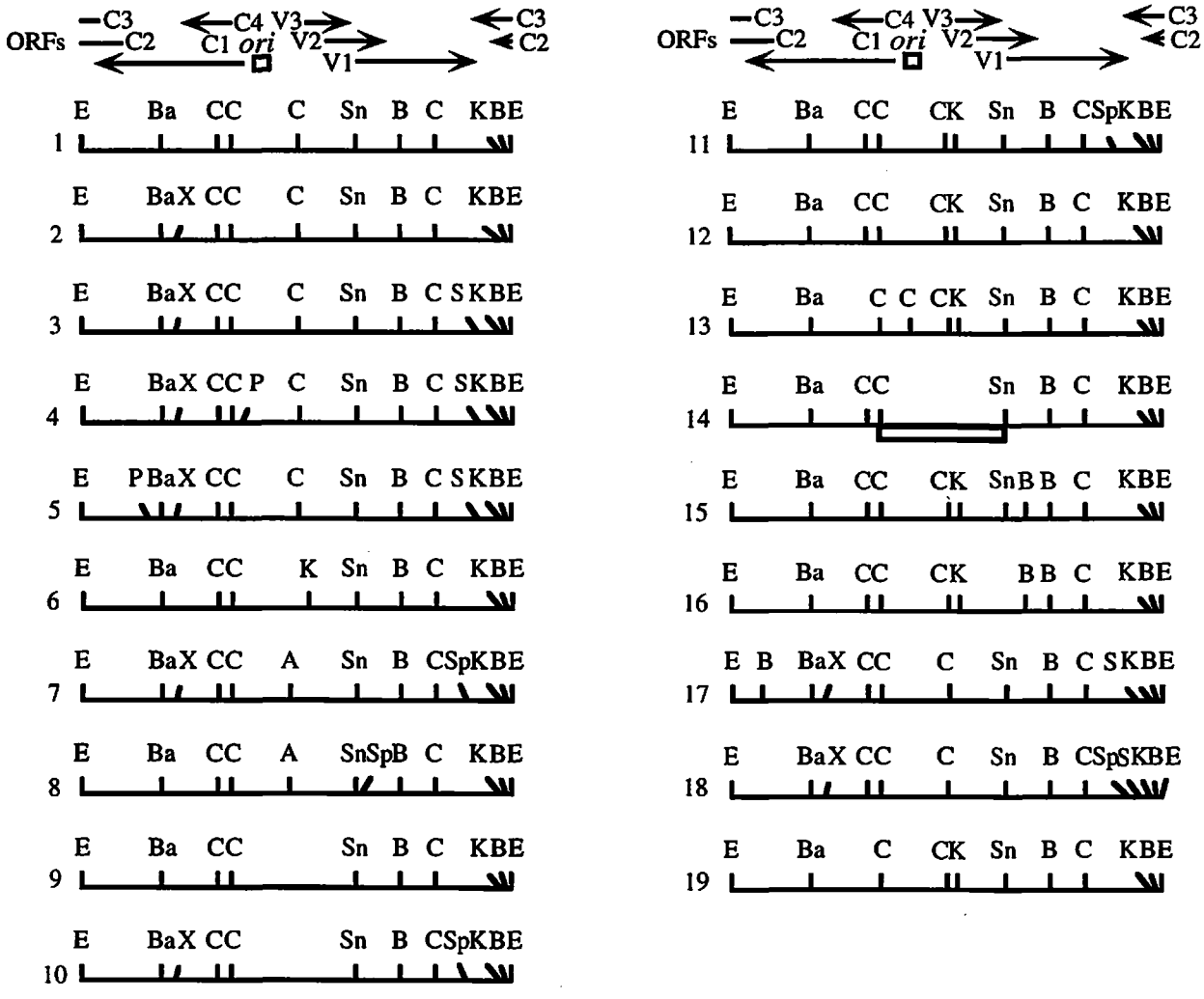

Fig. 2. Genotypic variants of BCTV-CFH. Presented are restriction endonuclease maps determined for cloned variants of the CFH strain of BCTV recovered from field, nursery, and laboratory isolates. Arrows denote locations and polarity of open reading frames (ORFs C1-C4, V1-V3) present on the sequenced isolate of CFH, and which are conserved among sequenced isolates of the Cal/Logan and Worland strains of BCTV. The location of *cis*-acting sequences of the origin of DNA replication (*ori*) is denoted by a box. Distinct genotypes are labeled by the number at left of each restriction map. Abbreviations for restriction enzymes are: A=*Apa* I, Ba=*Bam*H I, B=*Bst*X I, C=*Csp*45 I, E=*Eco*R I, H=*Hin*d III, K=*Kpn* I, P=*Pvu* II, Sa=*Sac* I, S=*Sal* I, Sc=*Sca* I, Sn=*Sna*B I, Sp=*Spe* I, Xb=*Xba* I, X=*Xho* I. The rectangle associated with genotype 14 denotes a region in which a deletion of 50-100 nucleotides appears to have occurred.

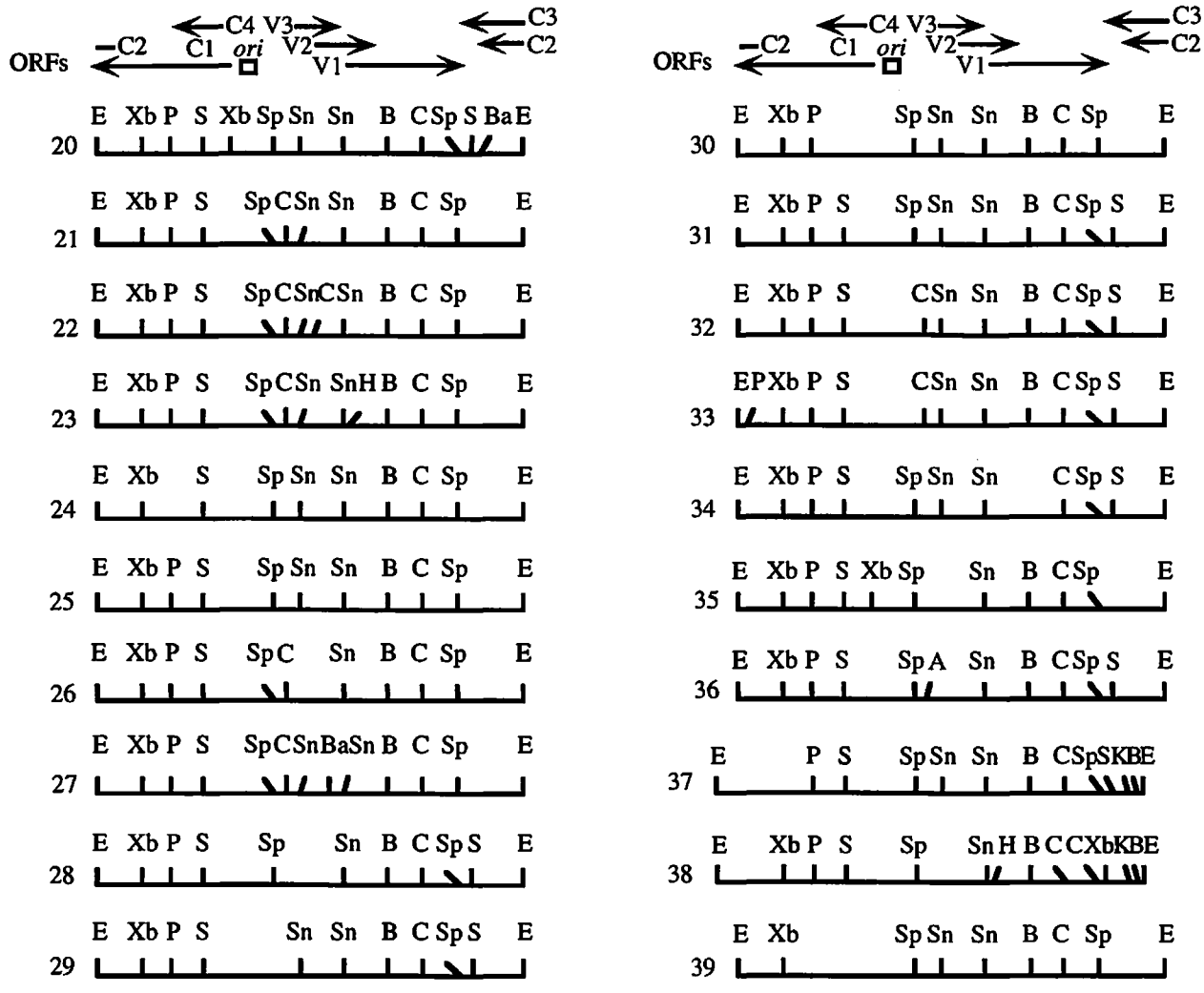

Fig. 3. Genotypic variants of BCTV-Worland. Presented are restriction endonuclease maps determined for cloned variants of the Worland strain of BCTV recovered from field, nursery, and laboratory isolates. Arrows denote locations and polarity of open reading frames (ORFs C1-C4, V1-V3) present on the sequenced isolate of Worland, and which are conserved among sequenced isolates of the Cal/Logan and CFH strains of BCTV. The location of *cis*-acting sequences of the origin of DNA replication (*ori*) is denoted by a box. Distinct genotypes are labeled by the number at left of each restriction map. Abbreviations for restriction enzymes are: A=*Apa* I, Ba=*Bam*H I, B=*Bst*X I, C=*Csp*45 I, E=*Eco*R I, H=*Hin*d III, K=*Kpn* I, P=*Pvu* II, Sa=*Sac* I, S=*Sal* I, Sc=*Sca* I, Sn=*Sna*B I, Sp=*Spe* I, Xb=*Xba* I, X=*Xho* I.

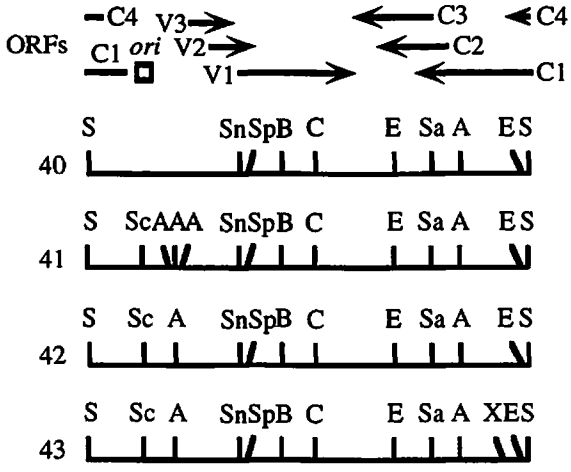

Fig. 4. Genotypic variants of BCTV-Cal/Logan. Presented are restriction endonuclease maps determined for cloned variants of the Cal/Logan strain of BCTV recovered from nursery and laboratory isolates. Arrows denote locations and polarity of open reading frames (ORFs C1-C4, V1-V3) present on the sequenced isolates California and Logan, and which are conserved among sequenced isolates of the Worland and CFH strains of BCTV. The location of *cis*-acting sequences of the origin of DNA replication (*ori*) is denoted by a box. Distinct genotypes are labeled by the number at left of each restriction map. Abbreviations for restriction enzymes are: A=*Apa* I, Ba=*Bam*H I, B=*Bst*X I, C=*Csp*45 I, E=*Eco*R I, H=*Hin*d III, K=*Kpn* I, P=*Pvu* II, Sa=*Sac* I, S=*Sal* I, Sc=*Sca* I, Sn=*Sna*B I, Sp=*Spe* I, Xb=*Xba* I, X=*Xho* I.

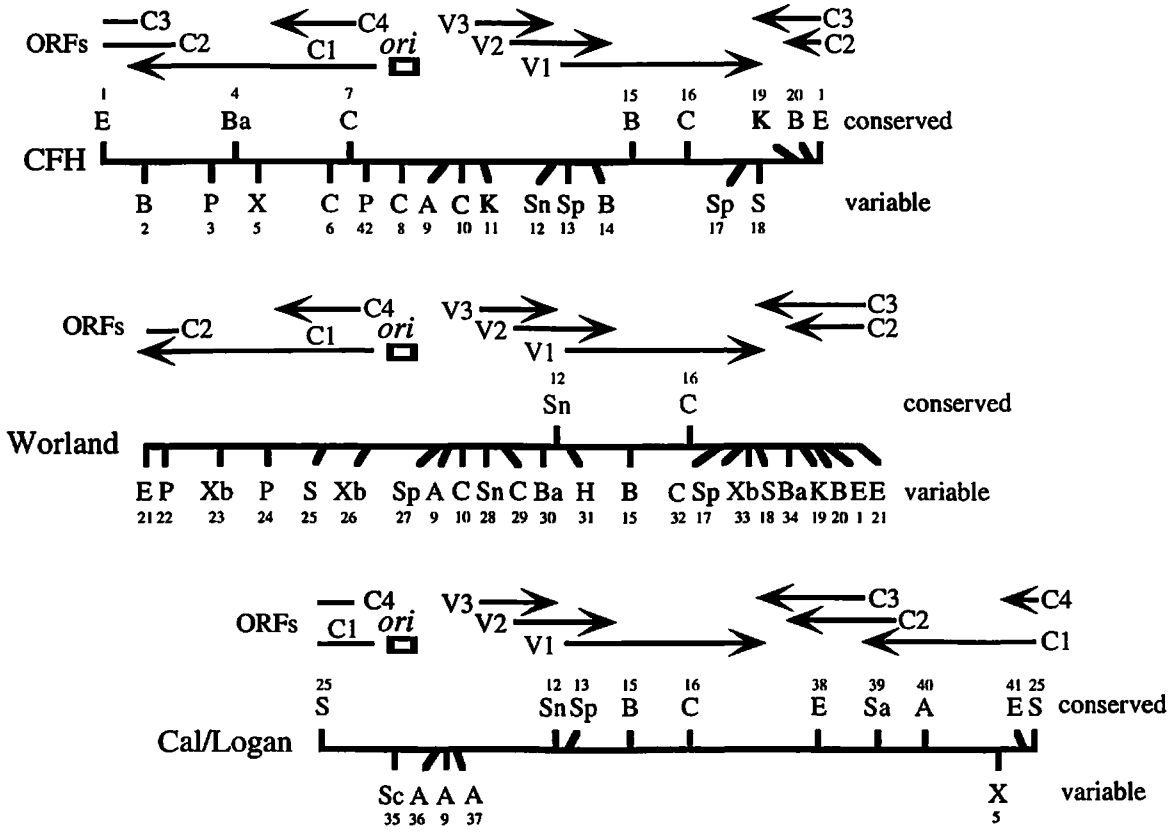

Fig. 5. Restriction site polymorphisms among strains of BCTV. Presented is an alignment of composite restriction maps of the CFH, Worland, and Cal/Logan strains of BCTV. Arrows denote locations and polarity of open reading frames (ORFs C1-C4, V1-V3) which are conserved among sequenced isolates of all three BCTV strains. The location of *cis*-acting sequences of the origin of DNA replication (*ori*) is denoted by a box. Restriction endonuclease sites conserved within a strain appear above, while sites which are variable within a strain appear below. Numbers identify each of 42 distinct restriction endonuclease sites mapped on one or more genotypes. Abbreviations for restriction enzymes are: A=*Apa* I, Ba=*Bam*H I, B=*Bst*X I, C=*Csp*45 I, E=*Eco*R I, H=*Hin*d III, K=*Kpn* I, P=*Pvu* II, Sa=*Sac* I, S=*Sal* I, Sc=*Sca* I, Sn=*Sna*B I, Sp=*Spe* I, Xb=*Xba* I, X=*Xho* I.

Table 1. BCTV field isolates and summary of cloning experiments.

Isolate	State	Locality	Representative Clone	Number of Clones	Strain	[c] Genotype
NC94-2	CA	Modesto	pNC94-2-175	3	CFH	15
CA95-1	CA	Firebaugh	pCA95-1-28	1	CFH	6
CA95-2	CA	Firebaugh	pCA95-2-52	2	CFH	6
CA95-4	CA	Firebaugh	pCA95-4-10	1	CFH	6
CA95-9	CA	Firebaugh	pCA95-9-181	1	CFH	16
CO95-1	CO	Wiggins	pCO95-1-4	2	Worland	34
			pCO95-1-13	2	Worland	31
CO95-3	CO	Wiggins	pCO95-3-27	3	Worland	38
			pCO95-3-41	1	Worland	36
CO95-6	CO	Ft. Morgan	pCO95-6-23	2	Worland	28
			pCO95-6-31	2	Worland	37
CO95-7	CO	Ft. Morgan	pCO95-7-35	1	Worland	35
CO95-8	CO	Ft. Morgan	pCO95-8-3	2	Worland	28
CO95-9	CO	Ft. Morgan	pCO95-9-2	2	Worland	28
CO95-10	CO	Ft. Morgan	pCO95-10-12	1	Worland	28
ID95-1	ID	Grandview	pID95-1-23	2	CFH	7
			pID95-1-149	1	CFH	10
ID95-2	ID	Grandview	pID95-2-1	1	CFH	11
			pID95-2-2	1	CFH	7
ID95-7	ID	Nampa	pID95-7-41	1	CFH	11
ID95-8	ID	Nampa	pID95-8-26	1	CFH	2
NM95-1	NM	Artesia	pNM95-1-94	1	CFH	17
NM95-2	NM	Artesia	pNM95-2-123	5	CFH	3
NM95-4	NM	Artesia	pNM95-4-99	2	CFH	3
[a]NM95-5	NM	Artesia	pNM95-5-28	1	Worland	28
[a]NM95-6	NM	Artesia	pNM95-6-49	1	Worland	29
			pNM95-6-60	1	Worland	30
[a]NM95-10	NM	Artesia	pNM95-10-7	1	Worland	25
			pNM95-10-21	3	Worland	31
[a]NM95-12	NM	Artesia	pNM95-12-3	1	Worland	32
			pNM95-12-18	1	Worland	33
OR95-3	OR	Jameison	pOR95-3-103	2	CFH	7
OR95-6	OR	Adrian	pOR95-6-74	1	Worland	23
OR95-7	OR	Adrian	pOR95-7-54	1	CFH	7
			pOR95-7-69	2	Worland	23
OR95-8	OR	Adrian	pOR95-8-17	2	CFH	7
OR95-9	OR	Adrian	pOR95-9-31	2	CFH	16
			pOR95-9-47	1	Worland	25

			pOR95-9-67	1	Worland	31
OR95-10	OR	Adrian	pOR95-10-106	1	Worland	23
[b] T94-1	TX	Bushland	pT94-1-25	5	CFH	4
[b] T94-4	TX	Bushland	pT94-4-59	6	CFH	3
			pT94-4-197	1	CFH	5
[b] T94-6	TX	Bushland	pT94-6-9	5	CFH	1
[b] T94-7	TX	Bushland	pT94-7-214	2	CFH	1
[b] T94-12	TX	Wildorado	pT94-12-87	3	CFH	1
[b] T94-14	TX	Wildorado	pT94-14-157	1	CFH	6
[b] T94-16	TX	Wildorado	pT94-16-17	4	CFH	1
[b] T94-19	TX	Wildorado	pT94-19-109	4	CFH	1
			pT94-19-154	1	CFH	2
[b] T94-23	TX	Wildorado	pT94-23-7	1	CFH	2
[b] T94-28	TX	Hereford	pT94-28-124	1	CFH	3
[b] T94-30	TX	Hereford	pT94-30-188	1	CFH	3
T95-1	TX	Bushland	pT95-1-114	1	CFH	18
T95-2	TX	Bushland	pT95-2-12	1	CFH	16
			pT95-2-49	1	CFH	18
T95-3	TX	Bushland	pT95-3-95	1	CFH	18
T95-4	TX	Bushland	pT95-4-13	4	CFH	18
WA95-1	WA	Mapton	pWA95-1-71	5	Worland	21
			pWA95-1-17	3	Worland	22
WA95-2	WA	Mapton	pWA95-2-47	4	Worland	23
			pWA95-2-192	1	Worland	24
			pWA95-2-221	1	Worland	25
WA95-3	WA	Mapton	pWA95-3-106	4	Worland	26
ML95-1	WA	Moses Lake	pML95-1-64	1	Worland	27
WD95-3	WY	Worland	pWD95-3-75	1	CFH	12
WD95-5	WY	Worland	pWD95-5-166	3	CFH	6
			pWD95-5-115	1	CFH	14
WD95-6	WY	Worland	pWD95-6-14	3	CFH	6
WD95-8	WY	Worland	pWD95-8-3	1	CFH	6
WD95-18	WY	Worland	pWD95-18-14	1	CFH	13
			pWD95-18-46	1	CFH	12
WD95-20	WY	Worland	pWD95-20-54	1	CFH	12
WY95-1	WY	Lovell	pWY95-1-93	1	Worland	28
			pWY95-1-95	1	Worland	23
			pWY95-1-101	2	Worland	31
WY95-2	WY	Lovell	pWY95-2-17	1	CFH	6
			pWY95-2-6	2	Worland	25
			pWY95-2-39	1	Worland	23
WY95-3	WY	Lovell	pWY95-3-62	1	Worland	23
WY95-5	WY	Worland	pWY95-5-8	1	Worland	25

pWY95-5-26	1	Worland	39
pWY95-5-35	1	CFH	12
pWY95-5-98	1	CFH	19

[a] Pepper isolates; all others are from sugar beet.

[b] Isolates and clones described previously in Stenger, 1995

[c] Genotype as defined by restriction endonuclease mapping as described in Figs. 2-4.

Table 2. Previously characterized laboratory and nursery isolates of BCTV.

Isolate	Origin	Representative clone	Strain	Genotype [a]	Reference
California	Modesto, CA	pBCTV028	Cal/Logan	40	Stanley, 1986
Logan	Logan, UT	pLOGAN	Cal/Logan	41	Stenger, 1990
CFH	Unknown	pCFH	CFH	1	Stenger, 1990
Worland	Worland, WY	pWORLAND	Worland	20	Stenger, 1990
BS94-1	Kimberly, ID	pBS94-1S-1	Cal/Logan	42	Stenger & Ostrow
		pBS94-1E-18	CFH	7	Stenger & Ostrow
BS94-3	Kimberly, ID	pBS94-3S-16	Cal/Logan	43	Stenger & Ostrow
		pBS94-3E-33	CFH	8	Stenger & Ostrow
		pBSDF94-3E-197	Worland	25	Stenger & Ostrow
BSDF94-1	Kimberly, ID	pBSDF94-1S-2	Cal/Logan	43	Stenger & Ostrow
		pBSDF94-1E-78	CFH	9	Stenger & Ostrow
BSDF94-6	Kimberly, ID	pBSDF94-6S-1	Cal/Logan	41	Stenger & Ostrow
		pBSDF94-6E-48	CFH	9	Stenger & Ostrow

[a] Genotype as defined by restriction endonuclease mapping as presented in Figs. 2-4.

Table 3. Occurrence of restriction endonuclease sites among genotypes of BCTV.

Site # [a]	Enzyme	Frequency (%) CFH (n=19)	Worland (n=20)	Calif/Logan (n=4)	All (n=43)
1	*Eco*R I	19 (100%)	2 (10%)	0 (0%)	21 (49%)
2	*Bst*X I	1 (5%)	0 (0%)	0 (0%)	1 (2%)
3	*Pvu* II	0 (0%)	1 (5%)	0 (0%)	1 (2%)
4	*Bam*H I	19 (100%)	0 (0%)	0 (0%)	19 (44%)
5	*Xho* I	8 (42%)	0 (0%)	1 (25%)	9 (21%)
6	*Csp*45 I	17 (89%)	0 (0%)	0 (0%)	17 (40%)
7	*Csp*45 I	19 (100%)	0 (0%)	0 (0%)	19 (44%)
8	*Csp*45 I	1 (5%)	0 (0%)	0 (0%)	1 (2%)
9	*Apa* I	2 (11%)	1 (5%)	3 (75%)	6 (14%)
10	*Csp*45 I	13 (68%)	7 (35%)	0 (0%)	20 (47%)
11	*Kpn* I	7 (37%)	0 (0%)	0 (0%)	7 (16%)
12	*Sna*B I	18 (95%)	20 (100%)	4 (100%)	42 (98%)
13	*Spe* I	1 (5%)	0 (0%)	4 (100%)	5 (12%)
14	*Bst*X I	2 (11%)	0 (0%)	0 (0%)	2 (5%)
15	*Bst*X I	19 (100%)	19 (95%)	4 (100%)	42 (98%)
16	*Csp*45 I	19 (100%)	20 (100%)	4 (100%)	43 (100%)
17	*Spe* I	4 (21%)	19 (95%)	0 (0%)	23 (53%)
18	*Sal* I	5 (26%)	9 (45%)	0 (0%)	14 (33%)
19	*Kpn* I	19 (100%)	2 (10%)	0 (0%)	21 (49%)
20	*Bst*X I	19 (100%)	2 (10%)	0 (0%)	21 (49%)
21	*Eco*R I	0 (0%)	18 (90%)	0 (0%)	18 (42%)
22	*Pvu* II	0 (0%)	1 (5%)	0 (0%)	1 (2%)
23	*Xba* I	0 (0%)	19 (95%)	0 (0%)	19 (44%)
24	*Pvu* II	0 (0%)	18 (90%)	0 (0%)	18 (42%)
25	*Sal* I	0 (0%)	18 (90%)	4 (100%)	22 (51%)
26	*Xba* I	0 (0%)	2 (10%)	0 (0%)	2 (5%)
27	*Spe* I	0 (0%)	17 (85%)	0 (0%)	17 (40%)
28	*Sna*B I	0 (0%)	15 (75%)	0 (0%)	15 (35%)
29	*Csp*45 I	0 (0%)	1 (5%)	0 (0%)	1 (2%)
30	*Bam*H I	0 (0%)	1 (5%)	0 (0%)	1 (2%)
31	*Hind* III	0 (0%)	2 (10%)	0 (0%)	2 (5%)
32	*Csp*45 I	0 (0%)	1 (5%)	0 (0%)	1 (2%)
33	*Xba* I	0 (0%)	1 (5%)	0 (0%)	1 (2%)
34	*Bam*H I	0 (0%)	1 (5%)	0 (0%)	1 (2%)
35	*Sca* I	0 (0%)	0 (0%)	3 (75%)	3 (7%)

36	*Apa* I	0 (0%)	0 (0%)	1 (25%)	1 (2%)
37	*Apa* I	0 (0%)	0 (0%)	1 (25%)	1 (2%)
38	*Eco*R I	0 (0%)	0 (0%)	4 (100%)	4 (9%)
39	*Sac* I	0 (0%)	0 (0%)	4 (100%)	4 (9%)
40	*Apa* I	0 (0%)	0 (0%)	4 (100%)	4 (9%)
41	*Eco*R I	0 (0%)	0 (0%)	4 (100%)	4 (9%)
42	*Pvu* II	1 (5%)	0 (0%)	0 (0%)	1 (2%)

[a] Site numbers as assigned in Figure 5.

Table 4. Genotypic complexity of BCTV field isolates [a].

Clones/isolate	Complexity	# of isolates	% of isolates (all)	% of isolates (clones >1) [b]
1	ND [c]	21	36	ND [c]
>1	1 genotype	17	29	46
>1	>1 genotype, 1 strain	16	28	43
>1	>1 genotype, >1 strain	4	7	11

[a] Based on 58 isolates described in Table 1.
[b] n = 37 isolates
[c] ND = not determined

Table 5. Occurrence of BCTV genotypes in field isolates [a].

		Frequency			
Genotype	Strain	Isolates	Clones	Localities	States
1	CFH	5	18	2	1
2	CFH	3	3	2	2
3	CFH	5	15	3	2
4	CFH	1	5	1	1
5	CFH	1	1	1	1
6	CFH	8	13	4	3
7	CFH	4	7	3	2
8	CFH	0	0	0	0
9	CFH	0	0	0	0
10	CFH	2	2	1	1
11	CFH	2	2	2	1
12	CFH	4	4	2	1
13	CFH	1	1	1	1
14	CFH	1	1	1	1
15	CFH	1	3	1	1
16	CFH	3	4	3	3
17	CFH	1	1	1	1
18	CFH	4	7	1	1
19	CFH	1	1	1	1
20	Worland	0	0	0	0
21	Worland	1	5	1	1
22	Worland	1	3	1	1
23	Worland	7	11	3	3
24	Worland	1	1	1	1
25	Worland	5	6	5	4
26	Worland	1	4	1	1
27	Worland	1	1	1	1
28	Worland	6	9	3	3
29	Worland	1	1	1	1
30	Worland	1	1	1	1
31	Worland	4	8	4	4
32	Worland	1	1	1	1
33	Worland	1	1	1	1
34	Worland	1	2	1	1
35	Worland	1	1	1	1
36	Worland	1	1	1	1

37	Worland	1	2	1	1
38	Worland	1	3	1	1
39	Worland	1	1	1	1
40	Calif/Logan	0	0	0	0
41	Calif/Logan	0	0	0	0
42	Calif/Logan	0	0	0	0
43	Calif/Logan	0	0	0	0
Total		84	150	60	53
Mean		1.95	3.49	1.40	1.23

[a] Isolates described in Table 1.